

**REMARKS**

Applicants wish to draw the Examiner's attention to the Disposition of Claims in the Office Action Summary on page one of the Office Action of 19 June 2003. Claims 1-37 are pending. Claims 8-33 are withdrawn.

Claims 1 and 2 are now amended with this Amendment. For the amendment to Claim 1, see page 6, lines 18-26 of the specification.

**Rejection of Claims 1-7 Under 35 U.S.C. § 102(b) (Item 3 of Office Action)**

Claims 1-7 have been rejected under 35 U.S.C. § 102(b), as they are said to be anticipated by Jean Amiral (US 5,466,582) as evidenced by Thorpe *et al.* (US 6,312,694).

Jean Amiral (US 5,466,582) describes a method to detect thrombocytopenia. In the method, a sample of patient plasma is mixed with a complex of heparin and platelet factor 4 (PF4), and the resulting mixture is tested to determine the presence or absence of antibodies to the complex. The complex to be used in the method of Jean Amiral can also be a heparin/platelet complex or a heparin/PF4/platelet complex. Jean Amiral does not describe a method using thrombospondin-1 (TSP-1) -- isolated, synthetic or recombinant -- in any way.

Claim 1 has been amended. The protein components of the complex of Claim 1, as amended, cannot be in platelets. Rather, the protein components are isolated from platelets or they are made by synthetic or recombinant means.

In the section of the Jean Amiral patent entitled "Aim of the Invention" (column 2, lines 36-50), it is stated:

"This novel technical solution is based on the detection, (i) by means of specific antigenic substance (Ag), (ii) of an antibody-type immunological material contained in the plasma of the subject to be tested and selected from the group consisting of anti(Y) antibodies (where Y is especially Z, or Z-Ag complexes) directed especially against the inductor drug Z and its complexes with Ag. In the light of the results of the work undertaken by the Applicant, it has been found that in warm-blooded animals, especially man and other mammals, the administration of a thrombopenia-inducing drug produces anti(Z), anti(Z-platelet), anti(Z-Ag) and anti(Z-Ag-platelet) antibodies leading to blood platelet aggregation or activation and causing thrombopenia."

In US 5,466,582, Jean Amiral described complexes as above in which Ag = PF4 and Z = heparin. However, Jean Amiral described no complexes including TSP-1. Jean Amiral did not incorporate TSP-1 into any complexes with heparin or PF4. It should be noted that a publication by Amiral *et al.* (*Thrombosis and Haemostasis* 73(1):21-28, 1995; Reference AR of Information Disclosure Statement) states the following on page 23, first paragraph: "The specificity of the type II HIT antibodies for PF4 was assessed as there was no antibody binding to the other heparin-binding proteins (i.e., ATIII, HCII, Vitronectin, Fibronectin, HRGP,  $\beta$ -Thromboglobulin, Thrombospondin, PDGF), in the presence or in the absence of heparin." Thus, one of skill in the art at the time of the invention had no reason to believe that TSP-1/heparin complexes or PF4/heparin/TSP-1 complexes could form in humans or could be made as isolated complexes.

The Examiner states, "Heparin binding proteins were isolated from mammalian blood during clinical trials (column 11, lines 10-35)." The lines of US 5,466,582 pointed out by the Examiner do not describe the isolation of heparin binding proteins from blood during clinical trials. Rather, the referenced lines describe the testing of patient plasma for antibodies (1) that bind to a heparin-PF4 complex, as described in Example 2 (2) that cause the aggregation of platelets in the presence of heparin. In the case of test (1), the only platelet protein used is isolated PF4. In the case of test (2), the platelet proteins remain on the surface of the platelet, and are not isolated, recombinant, synthetic or chimeric, as are the proteins of Claims 1-7. \*

The patent examiner stated, "Although Jean Amiral does not particularly point out what the platelet proteins are, evidence is provided by Thorpe *et al.* that TSP-1 and PF4 are found in platelet alpha granules and are known to associate with heparin." In the Thorpe *et al.* reference given by the patent examiner (US 6,312,694, col. 99, lines 47-50)), Thorpe *et al.*, did not state, and gave no evidence, that TSP-1 and PF4 are known to associate with heparin in a single ternary complex comprising heparin, PF4 and TSP-1 at the same time.

The Examiner seems to assume that Jean Amiral necessarily produced a ternary complex of PF4, TSP-1 and heparin. This is incorrect. Note column 6, line 43 through column 7, line 34, wherein the platelet factor 4 to be complexed with heparin is described. In all cases, the PF4 is recombinant, synthetic, complexed with proteoglycan, or in fractions containing proteins other than PF4, but in all cases the PF4 is purified away from TSP-1. The preparation of PF4

described in Example 1 of US 5,466,582 (column 9, lines 26-65) completely separates PF4 from TSP-1. Note that PF4 and TSP-1 elute from a heparin agarose column in fractions well separated in ionic strength.

The Examiner points out an assay kit as described by Jean Amiral, at column 10, lines 10-67 of US 5,466,582. The Examiner describes this assay kit as containing TSP-1. However, the referenced lines of US 5,466,582 make no mention of TSP-1 being a component of the kit. There is no reason to assume that TSP-1 is necessarily a contaminant of any other component of the kit.

Rejection of Claims 1 and 3-7 Under 35 U.S.C. § 102(b) (Item 5 of Office Action)

Claims 1-7 have been rejected under 35 U.S.C. § 102(b) "as being anticipated by Gogstad *et al.* (*British Journal of Haematology*, April 1983, vol. 53, (4), pages 563-73)."

Gogstad *et al.* describe crossed immunoelectrophoresis of solubilized platelets or subfractions of platelets against rabbit antibodies prepared against whole human platelets. For the detection of heparin-binding proteins, the lower half of the intermediate gel contained heparin immobilized on Sepharose 4B. When heparin-Sepharose 4B was included in the intermediate gel for crossed electrophoresis of solubilized platelets, several immunoprecipitates observed in the absence of heparin-Sepharose 4B were missing or covered a reduced area, indicating that the corresponding antigens were bound to the immobilized heparin in the second dimension electrophoresis. Binding to heparin-Sepharose 4B was shown by PF4 and by TSP-1.

Gogstad *et al.* do not teach a complex comprising heparin, PF4 and TSP-1. There is no evidence in Gogstad *et al.* that all three of these components can bind together in a soluble complex at the same time and under the same conditions.

what is difference?  
not true

The patent examiner stated that Gogstad *et al.* (*Br J Haematol*, 1983, 53: 563-573) teach a complex of platelet proteins that were isolated from alpha granules, and that these platelet proteins include PF4 and TSP-1 bound to immobilized heparin. This is not true at all. Gogstad *et al.* studied the platelet proteins that interact with heparin. In this work, Gogstad *et al.* solubilized platelet proteins in Triton X-100 and applied them to crossed immunoelectrophoresis against anti-platelet antibodies, using a medium in which an intermediate gel containing heparin covalently linked to Sepharose 4B was inserted. Gogstad *et al.* concluded that the platelets

contain at least six heparin-binding proteins which are present on the platelet surface or capable of being exposed to the extracellular medium after the release-reaction (of proteins from the platelet surface) has occurred. These proteins are PF4, TSP-1, GPIb and three proteins named in the Gogstad *et al.* paper as G4, 17 and 25.

The Gogstad *et al.* paper (*Br J Haematol*, 1983, 53: 563-573) does not discuss the possibility of a complex between heparin and any of the six mentioned proteins with which heparin can interact under some conditions (PF4, TSP-1, GPIb, G4, 17 and 25). Gogstad *et al.* do not discuss anything about heparin, PF4 and TSP-1 forming a ternary complex. Gogstad *et al.* teach that at least six platelet proteins can interact with heparin under some conditions but not, as was said by the patent examiner, that these proteins form a complex with heparin. There is no suggestion in Gogstad *et al.* that heparin can form a soluble complex with more than one protein at any one time.

Those of skill in the art who want to purify a heparin-binding protein (such as PF4 and TSP-1) from platelets or other cells, use heparin-Sepharose or heparin-agarose columns and initially allow the heparin-binding proteins to bind to the column. Proteins that do not interact with heparin are eluted from the heparin-Sepharose or heparin-agarose column by extensive washing at low salt concentration. Following the washing, a NaCl gradient buffer is applied to the column in order to elute the heparin-binding proteins.

Applicants demonstrated that heparin (not immobilized or linked with any support such as Sepharose 4B or agarose), TSP-1 and PF4 interact together and form soluble ternary complexes.

#### Rejection of Claims 34-37 Under 35 U.S.C. § 103(a) (Item 7 of Office Action)

Claims 34-37 have been rejected under 35 U.S.C. § 103(a) "as being unpatentable over Jean Amiral as evidenced by Thorpe *et al.* in view of Zuk *et al.* (US 4,281,061)."

The teachings of Jean Amiral (US 5,466,582) and the teachings of Thorpe *et al.* (US 6,312,694) have been discussed above. Zuk *et al.* (US 4,286,061) teach an immunoassay method and a kit in which predetermined amounts of the reagents for the immunoassay can be provided. Zuk *et al.* do not teach anything about the components of an assay for antibodies produced in heparin-induced thrombocytopenia (HIT) patients, and do not mention heparin, PF4 or TSP-1.

The Examiner states, "The teachings of Jean Amiral as evidenced by Thorpe *et al.* are set forth above and differ from the instant claims in not teaching all the components of a kit." It is true that the teachings of Jean Amiral as evidenced by Thorpe *et al.* do not include the components of the kits of Claims 34-37. Specifically, Jean Amiral does not mention any of these: a buffered medium comprising isolated human TSP-1 (Claims 34-36); a standardized positive control comprising known amounts of ternary complex reactive antibody (Claims 34-37); and a solid-phase support suitable for the immobilization of a platelet factor 4/heparin/thrombospondin-1 ternary complex (Claim 35). The teachings of Zuk *et al.* do not make up for the deficiencies of the Jean Amiral patent, as the Zuk *et al.* patent also does not teach any of these components of the kits of Claims 34-37.

As discussed above, the Jean Amiral patent does not teach that heparin, PF4 and TSP-1 form a PF4/heparin/TSP-1 ternary complex, nor does it teach the use of the PF4/heparin/TSP-1 complex as the antigen recognized by antibodies of HIT patients. The Jean Amiral patent teaches only a PF4/heparin binary complex.

One of ordinary skill in the art, studying the Jean Amiral patent, might want to carry out an assay for antibodies that bind to PF4/heparin complexes, as PF4/heparin complexes were thought to be the immunogen involved in HIT. Combining the teachings of the Jean Amiral patent with those of Zuk *et al.*, one of ordinary skill in the art might think of a kit containing reagents to produce PF4/heparin binary complexes and antibodies produced to PF4/heparin binary complexes. However, there is nothing in any of the references cited by the Examiner that would lead one of ordinary skill in the art to put together a kit that will detect the presence of immunoglobulin reactive with a PF4/heparin/TSP-1 complex, or a kit that would include TSP-1 in any way. One of ordinary skill in the art at the time of the invention would have had no reason to include TSP-1 in isolated complexes to be used in diagnosing HIT.

**CONCLUSION**

The Examiner is respectfully requested to consider the above amendments and remarks, and to withdraw the rejections. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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